

[3.0028]

PRELIMINARY STUDIES OF THE SPERMATOOZOA AND THE MALE  
REPRODUCTIVE SYSTEM OF SOME WHIRLIGIG BEETLES  
(COLEOPTERA: GYRINIDAE) <sup>1</sup>

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For some months several of us have been studying the developmental stages and the mature spermatozoa of various kinds of insects. During the course of this work, we have encountered an unusual situation in two species of whirligig beetles. This situation is described below.

As the spermatozoa of these gyrid beetles pass through the vasa deferentia, they become attached by their heads to rodlike objects which we have termed spermatostyles. These spermatostyles, each with more than 100 attached spermatozoa, also occur in the reproductive tract of the female. As yet we do not know the details relative to the formation of the spermatostyles and the association of the spermatozoa. However, because of the occurrence of cold weather which will limit our work for some months, and because the situation is so unusual, we feel that a report at this time is justified.

So far as could be determined, little work has been published on the reproductive systems of gyrid beetles. For this reason, a diagram of the male reproductive system is included, and a brief comparison is made between the reproductive systems of those beetles and those of a few related species.

Materials and Methods

The results reported here are based upon a study of two closely related species of gyrid beetles, *Dineutus assimilis* Kirby and *Dineutus carolinus* LeConte. These two species are very similar, and much of the work was done before we were able to distinguish between the two. So far no important differences in the reproductive system or in sperm development have been noted be-

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tween the two species. However, since both species were involved in the studies, the insects will be referred to as *Dineutus* or *Dineutus* sp. in the description and discussion to follow.

All specimens used were collected from ponds near Austin and San Marcos, Texas. Collections began near the first of July, 1969 and continued intermittently until low temperatures the middle of October presumably caused the beetles to disappear. During this period we collected and dissected several dozen beetles, and although no effort was made to collect males only, less than half a dozen females were found. The reason for the paucity of females is not apparent. In the laboratory, the insects were kept in plastic containers or aquaria containing pond water until they were dissected.

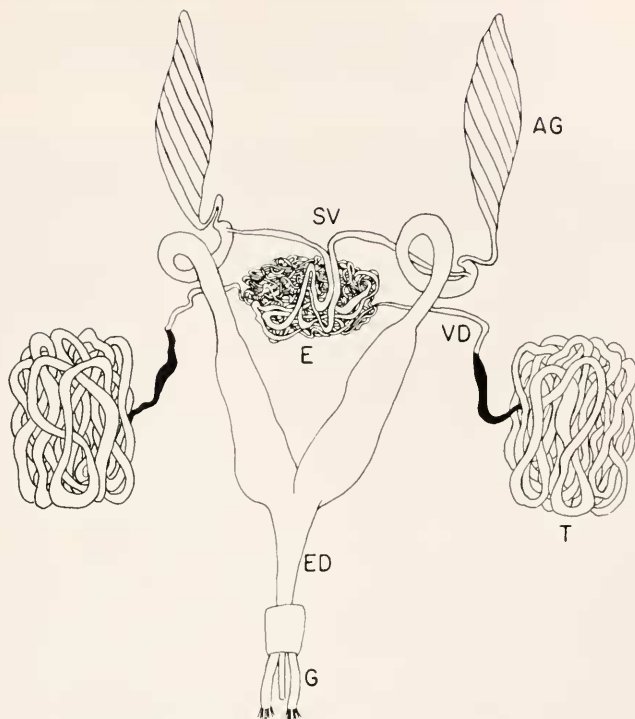
Sexes of the two species of *Dineutus* can be easily distinguished by grasping the beetles with blunt forceps, and examining the posterior abdominal sterna with a low power (e.g. 10X) dissecting microscope. The terminal sterna are somewhat transparent in strong direct light, and the external genitalia can be seen through them. The genitalia of males have three projections, whereas, those of females have only two.

The beetles were pinned to paraffin in a small dissecting dish, and dissected in Balar's saline using small scissors, fine needles and forceps. Portions of the reproductive system were placed in saline on a microscope slide and dissected further; a cover slip was applied and the material examined with a phase contrast microscope.

### Observations

A diagram of the male reproductive system of *Dineutus* sp. is shown in figure 1. This system is similar in many respects to that of *Dytiscus marginalis* (L.) (Dytiscidae) described by Blunck (1912) and Damandt (1912); and to two species of Dytiscidae examined by us, *Cybister* sp. and *Thermonectus* sp. There are, however, some differences. For example, in all the dytiscids the vasa deferentia join the ducts of the accessory glands very near the point of fusion of the accessory gland ducts into a single one. In *Dineutus* these connections occur considerably anterior to the fusion of the accessory gland ducts into a single duct. Also, in *D. marginalis* and *Cybister* sp. each vas deferens forms a separate epididymis, whereas, in *Dineutus* the two epididymides are entangled into a single mass. Only a single mass of epididymides occurs in *Thermonectus*.

The testes of *Dineutus* are single coiled tubes, not divided into follicles. The testes are thus similar to certain other species of Coleoptera, suborder Adephaga, that have been studied. In many insects, including some Coleoptera, suborder Polyphaga, the testes are composed of small divisions or follicles; the



**Figure 1.** Diagram of male reproductive system of *Dineutus* sp. AG, accessory gland; E, epididymides; ED, ejaculatory duct; G, external genitalia; SV, seminal vesicles; VD, vas deferens.

spermatozoa develop within these follicles and are often in cysts formed by testicular cyst cells (Blunck, 1912; Davey, 1965; Imms, 1960; Krause, 1946; Snodgrass, 1935; Williams, 1945).

Preliminary studies indicate that in *Dineutus* the spermatozoa do not pass through most of their development stages in cysts as often occurs in insects with testicular follicles. Single spermatozoa of varying lengths, with cytoplasmic blobs indicating immaturity, occur at the anterior ends of the uncoiled testes. As one proceeds posteriorly along the testes, the spermatozoa lose their blobs, become longer, and groups of spermatozoa become surrounded by cells which form cysts about each group. Near the posterior end of the testes, the walls of the cysts break and become dissipated. However, most of the sperm remain in groups, held together by masses of material at their anterior ends. These adhesive masses may be composed of cyst cells, or derived from these cells, as found

by Anderson (1950) in the Japanese beetle.

Within the vasa deferentia the groups of spermatozoa with the adhesive material became associated together into larger groups. Often chains of groups, considerably longer than individual sperm were seen, and the tails of the sperm were often twisted into tight spirals.

Each vas deferens passes from a testis toward the midline of the body, becomes very small and convoluted, and is entangled with the other to form a tightly coiled mass. The term epididymis (E, Fig. 1) is often used for a coiled or convoluted part of a vas deferens (Snodgrass, 1935; Tuxen, 1956). Because of the small diameter of the epididymides, and the compact tortuous coils, we have not been able to maintain complete orientation within this mass with conventional dissection methods. However, within the epididymides, groups of spermatozoa of varying lengths and spermatostyles have been encountered. Sometimes the spermatostyles had their complete complement of spermatozoa; sometimes only a few sperm were attached; and sometimes the rods were bare.

As indicated in the diagram, the very small tubes of the epididymides enlarge near their posterior ends forming what might be called seminal vesicles (SV, Fig. 1). These larger tubes are relatively easy to dissect. The formation of the spermatostyles and the attachment of the spermatozoa are essentially complete, so far as phase contrast microscopy reveals, by the time the seminal vesicles are attained. Well formed spermatostyles with attached spermatozoa also occur in the common ducts of the vasa deferentia and accessory glands, and they are also found routinely within the spermathecae of females. Spermatozoa, attached to spermatostyles, when removed from seminal vesicles, common ducts, or spermathecae, often became quite active in saline. In fact, on several occasions, the beating of the sperm tails has caused the spermatostyles to move rather rapidly through the saline. Detached spermatozoa were also often active and sometimes swam across the microscope field.

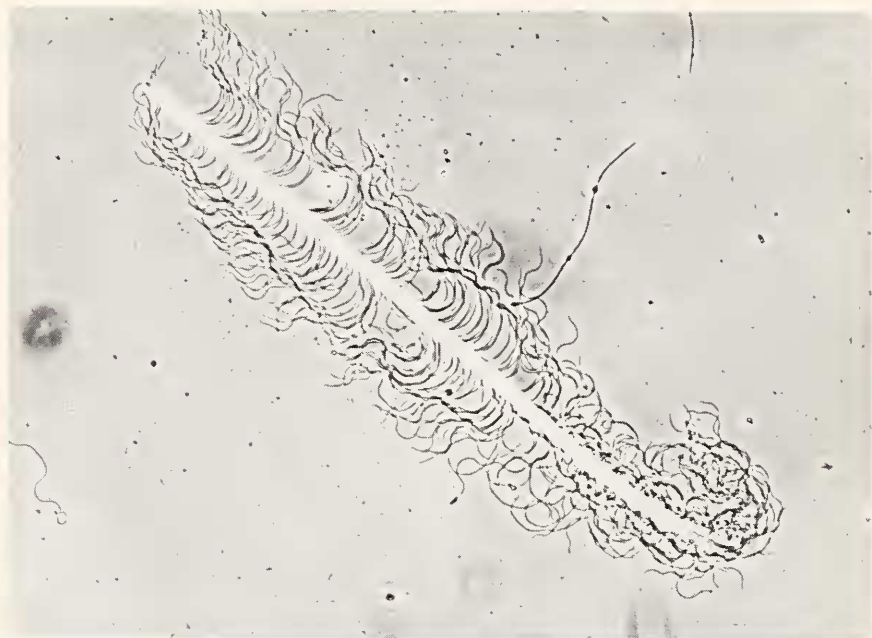
The rods or spermatostyles with their attached spermatozoa vary in length, diameter and conformation (Fig. 2 and 3) although it is probable that some of the variation may represent different developmental stages. We have tentatively assumed that most of the spermatostyles in the posterior part of the male reproductive system, and those in the female reproductive tract are fully developed. In these regions, most of the spermatostyles have been 800 to 1,000 micra in length, and the spermatostyles were apparently rigid (Fig. 3). In the epididymides, however, where formation of the rods and the association of the spermatozoa occur, much shorter spermatostyles have been found and some of the rods have been apparently flexible (Fig. 2). Individual spermatozoa are approximately 150 micra in length.

The development of the spermatozoa and the spermatostyles in these whir-

ligig beetles is apparently continuous during the summer in this area. From the first of July until the middle of October we averaged several dissections per week, and in practically all specimens we found similar stages in comparable parts of the male reproductive system.



Figure 2. Spermatostyles with attached spermatozoa, from epididymis of *Dineutus* sp. The curved, apparently flexible, spermatostyles suggest that they are not completely developed. Phase contrast, ca. 190X.



**Figure 3.** Spermatostyles with attached spermatozoa, from seminal vesicle of *Dineutus* sp. The relatively straight, apparently rigid, spermatostyle suggests that it is more completely developed than those in Fig. 2. Phase contrast, ca. 225X.

### Discussion

The observations we have made to date suggest two possibilities with respect to the formation of the rods or spermatostyles, and the attachment of the spermatozoa. One possibility is that the rods are formed first, and the spermatozoa attach to the spermatostyles after they have formed. We have seen rods free of spermatozoa in various parts of the vasa deferentia, but we have not seen anything free of spermatozoa that could be considered as developmental stages of the rods. All spermatostyles free of spermatozoa we have seen have been fully developed. There has not been any indication that individual motile sperm are attracted to the spermatostyles, nor can we suggest a method by which the spermatozoa could attach to a fully formed rod. For these reasons, and for other reasons to be mentioned, we are at present inclined to support the second suggested sequence of events noted below.



We have mentioned that groups of spermatozoa with their anterior ends embedded in masses of material (possibly cyst cells) occur near the anterior ends of the vasa deferentia. It has been noted also that these groups occur attached to each other in chains of varying lengths. Shiny material, similar in appearance to that at the anterior ends of the individual bundles can often be seen along these chains, and each bundle is probably attached to the chain by this material. It is suggested that as development proceeds that the adhesive material becomes transformed into the rods or spermatostyles (possibly with some contribution from the epididymides) with the heads of the spermatozoa embedded within them. What might cause this transformation has not been established, although certain things are suggestive. Anderson (1950) found evidence that cyst cells are capable of active synthesis, and the material in which the groups of sperm heads of the gyrinid beetles are embedded is probably composed of cyst cells or is derived from the cells. Since the sperm tails within the groups of sperm often occur in tight spirals, it seems possible that the tails could help in compressing the material into the rods.

It is possible that the spermatostyles with their attached spermatozoa could be considered as modified spermatophores, but so far as could be determined, nothing comparable to the present situation has ever been reported in insects. Spermatophores that have been described vary considerably in structure, method of formation, and time of formation (cf. Alexander, 1963; Davey, 1960; Hinton, 1963; Khalifia, 1949; Wilde, 1964). In some cases they are elaborate structures consisting of sperm sacs in a gelatinous matrix; in other instances either the matrix or the sperm sacs are lacking. Hyaline threads or rods supporting droplets containing sperm (some *Collembola* and *Thysanura*) are considered by some to be modified spermatophores (e.g. Alexander, 1963). In some insects the spermatophores are completely formed in the male before copulation; in others they are formed during copulation, and partly within the body of the female. The origin of the gelatinous matrix is not known in all cases, but sometimes it is formed by the male accessory glands and/or cells in the vasa deferentia. The hyaline rods in the *Collembola* and *Thysanura* mentioned above may be similar to the spermatostyles, but in these cases the spermatozoa are apparently not tightly attached to the rods as in the gyrinids, and the methods of transfer from male to female are presumably different in the two groups.

Although it cannot at present be determined whether or not the spermatostyles and their attached sperm are homologous with spermatophores, one similarity between the two is obvious. The combination of spermatostyles and spermatozoa makes it possible for large numbers of sperm to be transferred to the female at one time, an occurrence which might be considered as one function of a spermatophore. Indications are that within the spermatheca of

the female the spermatozoa become detached and the spermatostyles eventually disintegrate. Broken and partially disintegrated spermatostyles, free of spermatozoa, have been recovered from spermathecae on two occasions.

It seems possible that certain phenomena reported in the Dytiscidae may have some significance with respect to the present situation. It has been known for a long time that in some Dytiscidae the spermatozoa occur in pairs with the heads fused together forming what have been called double sperm. During development in these insects, masses of tightly wound spermatozoa occur with their heads bound together by an attachment mass (the so-called *Klebmasse*); in some species, chains of spermatozoa are formed and small rodlets may develop from the attachment mass (Ballowitz, 1895). According to Ballowitz, the heads of double sperm and the chains of sperm are held together by material from the attachment mass. Several of the illustrations published by this author are very similar to certain developmental stages we have seen in *Dineutus*.

Sharp and Muir (1912) found that the genitalia of the Gyrinidae are more similar to the Hydrophilidae than to the Dytiscidae, but most modern workers believe that the Gyrinidae and Dytiscidae are closely related (e.g. Arnett, 1963; Crowson, 1960; Leech and Chandler, 1956). It has been suggested also that modern gyrimids and dytiscids are descended from dytiscid stock (Crowson, loc. cit.). The similarities in the male reproductive systems of *Dineutus* and several species of dytiscids mentioned previously, as well as, the similarities of sperm developmental stages, could be considered as additional evidence that gyrimids and dytiscids are closely related. Perhaps both modern gyrimids and dytiscids inherited a rudimentary pattern of unusual sperm association and some gyrimids further elaborated this pattern to form the rods or spermatostyles.

One additional point should be mentioned. The large size and apparent complexity of the accessory glands in *Dineutus* suggest that these glands have an important function in reproductive processes. In fact our first thought when the spermatostyles were discovered was that the spermatostyles were formed by the accessory glands. However, we have stated previously that the spermatostyles are apparently completely formed, and the spermatozoa are associated with them before the vasa deferentia join the ducts of the accessory glands. This situation suggests that the accessory glands do not have a major role in the formation of the spermatostyles. It seems possible, however, that the accessory glands may contribute additional material to the spermatostyles after they attain the common duct of the vasa deferentia and the accessory glands. It also seems probable that the accessory glands secrete material which could help in the transfer of the spermatostyles to the female.

Additional studies of this unusual situation, including electron microscopy, are now in progress.



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**2.0028 Preliminary studies of the spermatozoa and the male reproductive system of some whirligig beetles (Coleoptera: Gyrinidae).**

Abstract.— Studies have been made of the developing spermatozoa and the male reproductive systems of two species of whirligig beetles, genus *Dineutus*. As the spermatozoa of these beetles pass through the vasa deferentia, they become attached by their heads to long rods (as much as 1,000 micra in length) which have been termed spermatostyles. These spermatostyles with the attached spermatozoa also occur in the female reproductive tract. Two possible methods of formation of this unusual sperm association are discussed. Brief comparisons are made between the reproductive systems of *Dineutus* and some Dytiscidae, as well as, comparisons of sperm associations in the two groups.— Osmond P. Breland and Everett Simmons, Department of Zoology, University of Texas, Austin, Texas 78712.

*Descriptors:* Coleoptera; Gyrinidae; spermatozoa; male reproductive system; *Dineutus* spp.; spermatostyles; Dytiscidae.